

PHYSIOLOGICAL RACES OF
Belonolaimus longicaudatus, RAU

By

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INTRODUCTION

The sting nematode, Belonolaimus longicaudatus Rau is recognized to be a pathogen of many crops in Florida and in certain other states of the Southeastern U.S.A. The host list is long with most flowering plant families represented. These include such important plants as corn, turf grasses, citrus, cotton, peanut and most vegetables cultivated in Florida. Nematicides are extensively used for control of this pest in fields used for the production of vegetable and agronomic crops, on golf course greens and fairways and in home lawns. Most scientific studies with sting nematodes have dealt with host-parasite relations, control or taxonomy and morphology.

Some interesting facts have been revealed to indicate the possibility that physiologic races may occur within the species B. longicaudatus. Owens (62, 63) reported it to be a pathogen of peanuts and that was verified by Christie et al. (13) and others. Sasser and Cooper (77) attributed great losses in production of peanuts to the widespread occurrence of the sting nematode in the peanut growing belt of the Southeastern U.S.A. Yet no reports have been published on losses of peanut production in Florida and Georgia due to parasitism of this pest. In fact, Perry and Norden (67) reported that a population of B. longicaudatus in a field near Gainesville, Florida "failed to attack peanut".

This study was initiated then to determine if different physiologic races of the parasite do exist and to investigate the possibility of

morphological differences. Three populations of the pest were collected from Gainesville, Sanford, and Fuller's Crossing, all in Florida. These sites were chosen because the Gainesville population was reported not to attack peanut. The Fuller's Crossing population had been reported to attack citrus, an unknown host for the other two populations.

REVIEW OF LITERATURE

In 1942, Steiner (88) described the genus Belonolaimus, with B. gracilis as the type species, from specimens collected from about the roots of slash pine seedlings growing near Ocala, Florida, U.S.A. Rau (70), in 1958, described B. longicaudatus from soil and roots of corn collected at Sanford, Florida. He distinguished B. longicaudatus from B. gracilis primarily by longer tail and shorter stylet of females of the former. Rau claimed in 1961 (71) that B. longicaudatus is the predominant species, and provided emended descriptions of both species. Goodey (28), in 1963, proposed that B. longicaudatus be placed in synonymy with B. gracilis. However, most workers recognize B. longicaudatus as a valid species. Rau (71) stated that in his opinion, the research done by various authors following Steiner's description actually dealt with B. longicaudatus rather than B. gracilis. Examination of illustrations by certain workers and specimens obtained from numerous locations indicate that this is true. Actually a close examination of the illustrations by Steiner and Rau indicate that B. gracilis Steiner and B. gracilis Rau are not the same species.

In 1963, Rau (72) described B. euthychilus from around roots of Pinus palustris Mill. growing in Florida, B. martimus from around the roots of Uniola paniculata L. growing in Florida, and B. nortoni from about the roots of Zea mays L. growing in Texas. In 1960 B. hastulatus was described from Queensland, Australia by Colbran (15) from soil around the roots of a Late Valencia orange on sweet orange rootstock.

He distinguished the species primarily by the presence of four sub-lateral incisures on each side of the body and a relatively short stylet. Roman (75), in 1964, added another species B. lineatus characterized also by the presence of four lateral lines in the lateral field. Sauer (79) transferred B. hastulatus to a new genus Morulaimus Sauer 1965, and proposed keeping B. lineatus under Belonolaimus.

The symptomatological effects of sting nematode have been described for various host plants by several workers (11, 13, 45, 62, 63, 73, 76, 80, 86, 87). The above-ground symptoms generally involve those of stunting and chlorosis. These symptoms are much the same as those brought on by any factor which prevents normal root growth and functions. Christie et al. (13), Christie (11) and others reported that sting nematodes feed on or near root tips. The root tips are devitalized and apparently mitosis is stopped resulting in the "stubby root" ?? condition reported for most hosts. Some necrosis on the roots may also occur. Holdeman and Graham (36) reported that minute dark, shrunken lesions appeared along the axis of cotton roots soon after being parasitized by sting nematodes. These lesions at times advanced laterally and girdled the roots which often broke off. High populations on peanuts also resulted in minute dead areas on the roots (50).

Histological studies were conducted to determine the pathological effects of B. longicaudatus on grapefruit roots by Standifer and Perry (87) and on bean roots by Standifer (86). Essentially both studies showed that "feeding of the sting nematode results in a lesion consisting of a cavity surrounded by affected cells characterized by ruptured cell walls, coagulated protoplasm, and altered staining reactions." Lesions occurring at or near root apices resulted in maturation of root tissues virtually to the apices.

<u>Vegetable</u> <u>crops</u> (contd.)	Fla.	Ga.	S. C.	N. C.	Va.	Ala.	La.	Tex.	Ark.	N. J.	Conn.	Bermuda
Peas	(70)											
Peppers	(70, 85)	(31, 85)										
Potato	(31, 70)	(35)										
Sweet			(4, 32, 35, 85)		(85)							
Radish	(70)											
Squash	(31, 58, 85)	(85)	(35)									
Tomato	(58, 70)	(85)	(35)									
<hr/>												
<u>Field</u> <u>crops</u>												
Alfalfa		(85)										
Barley			(85)									
Clover												
Corn	(6, 7, 13, 31, 58, 64, 66, 70, 88)	(31, 85)	(1, 29, 30, 31, 35)	(18, 19, 71, 77)	(31, 63)			(85)				(40)

Field crops (contd.)	Fla.	Ga.	S.C.	N.C.	Va.	Ala.	La.	Tex.	Ark.	N.J.	Conn.	Bermuda
Cotton	(70)	(25, 31) (18, 18, 31, 33, 19, 31) 34, 36)	(18, 18, 31, 33, 19, 31) 34, 36)	(18, 18, 31, 33, 19, 31) 34, 36)	(18, 18, 31, 33, 19, 31) 34, 36)	(50, 51, 53)						
Hairy Indigo	(58)											
Lupines		(31)										
Millet		(31)										
Oat			(35)									
Peanut	(70)	(31)	(18, 18, 31, 33, 19, 31) 34, 36)	(17, 18, 19, 77, 78)	(31, 49, 62, 63)	(50, 53)						
Rye			(35)									
Sesbania	(59)											
Sorghum	(60)											
Soybean	(13, 70, 94)	(18, 18, 32, 77)	(18, 18, 32, 77)	(31, 63)								
Sugarcane												
Vetch		(27)										

(46)

(74)

Grasses	Fla.	Ga.	S.C.	N.C.	Va.	Ala.	La. Tex.	Ark.	N.J.	Conn.	Bermuda
Bahiyagrass	(58, 61, 85)	(85)									
Bermuda-grass	(24, 31, 61, 70, 85, 91)	(26, 85)	(1, 35)		(85)	(85)					(65)
Centipede-grass	(24, 31, 70, 85)	(28, 85)	(1)								
Crabgrass		(85)	(35)								
Fescue			(35)								
Highland bent					(85)						
Pangola-grass	(58)										
St. Augustine grass	(24, 31, 61, 70, 73, 85)	(28, 85)									
Sudan-grass		(31)	(31)		(35)						
Unspecified turf	(12, 24, 43, 54, 59, 66, 84, 91)		(24, 84)	(84)		(50, 85)				(37)	

35
144
5

Grasses
 (contd.)
 Zoysia-
 grass

(24, 61,
 70) (26, 85)

Trees and
 Shrubs

Boxwood

(85)

Citrus

(5, 21,
 22, 54,
 70, 85,
 87, 88)

Dogwood

(85)

Grape

(12, 80) (N)

Oak

(85)

Peach

(85)

Pine

(38, 85,
 88)

Rose

(85) (81)

Strawberry

(6, 7,
 13, 31,
 70, 85) (85) (30, 34)

Sycamore

(76)

Youpon

(85)

Yew

(85)

Distribution of the sting nematode is considered to be limited by soil texture. In a greenhouse test, Thames and Thornton (93) found that the largest number of specimens were recovered from pots of soils containing 3.2% clay. The numbers recovered decreased as the clay content increased. Thames (92) examined 762 soil samples from South Florida and concluded that B. longicaudatus was unlikely to increase to large populations in soils of textures finer than sandy loams. Christie (10) in 1953 pointed out that although the sting nematode is fairly widespread in Florida, it has never been found in the muck or marl soils.

In 1967, Potter (68) in North Carolina studied the vertical distribution of B. longicaudatus following peanuts between November and April, 1966. He found that larval population density was highest at a depth of 15.2 - 30.4 cm and least at 0 - 15 cm. He suggested that overwintering of the nematode may take place at 30.4 - 45.6 cm. Also in North Carolina Barker (3) studied the population dynamics of the sting nematode on a monthly basis for one year. He found that the greatest numbers of the nematode were recovered from the 7.5 - 15 cm depths in October through January. Relatively high numbers were also recovered during the fall season at 0 - 7.5 cm and 15 - 30 cm depths, whereas very few were recovered from 30 - 45 cm depths at any time. He also reported that the nematode virtually disappeared at all depths in March and April and increased slightly in May and June.

In 1952, Holdeman and Graham (33) reported for the first time a nematode-diseased complex involving the ectoparasitic nematode B. longicaudatus and Fusarium wilt of cotton. They and others (34, 37, 51) concluded that Fusarium-resistant cotton varieties succumbed to

the disease, and young seedlings of susceptible cotton varieties were severely damaged when both organisms were present. Cooper and Brodie (16) found that cotton varieties in which root-knot resistance contributed to wilt resistance, were also resistant to the sting nematode-Fusarium complex.

In 1953, Raski (69) discussed the significance of analyzing leaves, stems, and roots of plants for their mineral contents. He suggested that this analysis may assist in determining how nematodes affect plants and also such procedures might prove helpful in the detection and diagnosis of nematode problems.

In 1893, Vanha (95) analyzed the dry matter of sugar beet nematode infected beets and found a lower percentage of K, P, Mg, and Ca. The work of Wilfarth and Wimmer (96) in 1903 showed that nematode infected beets had lower percentages of N, K, Na, Ca and Mg than the healthy ones. Kruger (44) and Neuworth (52) concluded that the sugar beet nematode does not damage the absorption ability of the root system, but does damage the beets by depriving them of their nutritional substances.

Much work has been done on the effects of the root-knot nematode Meloidogyne spp. on the nutritional status of tomatoes. Oteifa (55) in 1952 showed that M. incognita infected tomato plants had lower amounts of N, P, Ca, Mg, and K as compared to control plants. He also noted that the rate of absorption of these elements was affected by the presence of the nematode. In tomato roots heavily infected by M. incognita acrita, Maung (47) and Maung and Jenkins (48) found significantly higher rates of N, P, and K than in the control plants. However, Na accumulation was significantly lower in moderately and

heavily infected roots. Bergeson (4) arranged split rooted tomato plants with each half root system grown in a separate pot. Both systems were infected with M. incognita. One root system received fertilizer (feeding roots), while the other did not (non-feeding roots). He concluded that the high increase in N and K in infected-non-feeding roots was due to "...mobilization of the minerals to the infection site and this mechanism is probably more important in causing mineral excess in galled roots than in mechanical blockage of the vascular system."

Nutrients containing labeled phosphorus (P^{32}) were used by several investigators for determination of mineral absorption, accumulation and translocation in root-knot nematode-infected tomato plants. Dropkin and King (20) showed that P^{32} accumulated at a lower rate in the galled tissue of tomato roots infected with M. incognita acrita than in uninfected roots of the same plant. They also found that less P was translocated out of the galled roots than out of the ungalled roots. Hunter (39) concluded that the total percentage of P^{32} contents was larger in the M. incognita acrita-infected tomato plants than the controls, but found no decrease in the rate of translocation of P^{32} or the amount of translocation of any other element. Oteifa and Elgindi (56, 57) found that galled and ungalled tissues of the same M. javanica-infected tomato roots were capable of P^{32} absorption, but accumulation in galled tissue always significantly exceeded the amount of P^{32} incorporated in the ungalled tissue. With an increase in the duration of infection, they found an increase in the amount of P^{32} that accumulated in the root tissues and a decrease in that translocated to the shoot tissues.

Chitwood et al. (9) in 1952, discussed the association of M. Incoqnita and M. javanica with decline and chlorosis of peach trees. He suggested that clorosis "...appeared to be due to deficiency of Mg or Fe or both." In resistant varieties, he found lower rates of Ca and Fe, but K usually showed an increase regardless of specific nematode or degree of resistance or susceptibility of peach seedlings.

Tarjan (90) analyzed leaves and roots of English boxwood infected with Pratylenchus spp. When compared with healthy plants, roots of infected plants contained higher levels of Na and N, and lower levels of K. Chevres-Roman (8) showed marked increases in the percentage of inorganic N and K in plant and soil when sorghum and corn were heavily parasitized by either Trichodorus porosos, Tylenchorhynchus claytoni or Pratylenchus zeae. He also demonstrated that parasitized plants removed much less N and K from the soil than healthy plants, and suggested plant-parasitic nematodes cause "...inefficient utilization of nutrient elements and water...." Jenkins and Malek (42) inoculated vetch plants with several nematodes and found that M. hapla induced the greatest changes in the root tissue, where N was the most affected. Trichodorus christiei, on the other hand induced the greatest changes in the shoot tissues where K was the most affected.

Very little work has been done on the influence of plant-parasitic nematodes on the nutritional levels of citrus trees. Feldman et al. (23) analyzed leaves from healthy and citrus trees diseased with Radopholus similis. They found that leaves from diseased trees were lower in both K and N than were leaves from healthy trees. The lowest K concentration was in the visibly declined trees, but low concentrations of K existed in the first 2-3 rows of the apparently healthy trees. They found no difference between levels of P in declined or healthy trees.

In 1952 Christie et al. (13) reported that corn and strawberry plants showed a chlorotic condition when parasitized with B. gracilis (B. longicaudatus). They proposed that this condition was perhaps due to Fe deficiency. However, they stated "...apparently, when the plants are growing in soil in which some essential element is available in very limited amounts, nematode injury to the roots may cause, indirectly, the appearance of deficiency symptoms on the foliage...."

MATERIALS AND METHODS

Parasitism Tests

Three populations of Belonolaimus longicaudatus were collected from the following locations: 1) a corn field at the Tobacco Unit, Florida Agricultural Experiment Station, Gainesville, Florida; 2) corn inoculated at the Central Florida Experiment Station but originally the inoculum came from a field near Sanford, Florida; 3) and a citrus grove near Fuller's Crossing, Florida. Populations were increased by filling individual greenhouse flats with one of the three infested soils and then planting each of them to sweet corn, Zea mays L. 'Silver Queen' and bean, Phaseolus vulgaris L. 'Contender'. Two rough lemon seedlings, Citrus jambhiri Lush., were planted in the flat containing the Fuller's Crossing soil about one month later.

Rough lemon, Citrus jambhiri Lush.; peanut, Arachis hypogaea L. 'Early Runner'; strawberry, Fragaria hybrid 'Florida 90'; and tomato, Lycopersicon esculentum Mill. 'Rutgers' were tested as hosts for each of the three populations of sting nematodes. A Kanapaha fine sand soil was obtained from a field of the Florida Agricultural Experiment Station. The soil was fumigated with methyl bromide at the rate of 2 lb/27 cu ft and then aerated for a minimum of 4 weeks. The soil was then used to fill the number and type of greenhouse pots required for each experiment.

The nematodes used as inoculum were isolated from the soil by using the Seinhorst elutriator method (82). Inoculations were made within

12 hours after the nematodes were isolated. Each pot of soil to be inoculated received 100 specimens (90 females and 10 males). The nematodes were hand-picked with a bamboo pick, and placed in a Syracuse dish containing distilled water. The nematodes then were transferred with a dropper to a shallow depression made either in the center of the soil surface (peanut), or around the roots of seedlings (rough lemon, strawberry, and tomato). The depression was then filled with soil and watered with gentle spray. Treatments were replicated three or six times and equal numbers of uninoculated plants were maintained as controls. The pots then were placed in either a greenhouse or a plant growth room, wherein the temperature was maintained at approximately 80° F. The plants were fertilized as required for good growth.

At the end of each experiment, a predetermined quantity of soil was taken from each pot, and processed for nematode counts using the Christie and Perry method (14). Tabulated data from all pathogenicity tests were statistically analyzed by a computer.

Parasitism on Rough Lemon

Rough lemon seeds were allowed to germinate in vermiculite, and when the seedlings were about 25 cm in height, 24 seedlings of a uniform size were chosen. The root systems were washed with tap water, and one seedling was transplanted into each of 24 six-inch autoclaved clay pots containing 1,000 ml of sterilized soil. The nematodes were added about 3 weeks later on January 30, 1967. Each treatment was replicated six times in a randomized block design.

Within about 6 months, differences between the various treatments became apparent. At this time each plant was removed from the pot, the roots were carefully extracted from the soil, washed and partially

dried on paper towels. The weight of each plant (root and shoot) was recorded and a 100 ml sample of the soil from each pot was processed for nematode counts. Each seedling then was replanted in a 2-gal- porcelain crock containing the original 900 ml of soil plus 1,600 ml of sterilized soil (total 2,500 ml).

The experiment was terminated after about 14 months on April 3, 1968. The roots were removed from the soil, washed, surface dried with paper towels, and weighed. A portion of each root system was dried in a drying room set at 70° C and retained for mineral determinations. The foliage portion of each plant was weighed, and ten leaves were randomly selected from below the 4 terminal leaves, dried and assayed for mineral determinations. Data on height, number of leaves, and diameter of each stem near the soil surface were recorded. The soil from each crock was thoroughly mixed and a 250 ml sample was processed for nematode count. One plant which was inoculated with the Sanford population died because of poor drainage and was discarded. Values of the missing data were accounted for in the computations.

Parasitism on Peanut

Peanut seeds were allowed to germinate in the laboratory on moist paper towels until sprouts were about 2 inches long. Clay pots of a six-inch size were autoclaved and filled with 1,000 ml of sterilized soil. A shallow depression was made in the middle of each treated pot and on January 8, 1967, two sprouted seeds were placed in the depression and 100 specimens of the respective inocula immediately were poured onto the sprouts. The depression was carefully filled with soil and water was added. Each treatment was replicated six times and the pots were arranged in a randomized block design.

These pots were taken down after 5 months on June 3, 1967. Weight of roots and tops, and number and weight of nuts were recorded. A 100 ml sample of soil from each pot was processed for sting nematode counts. The remaining soil (900 ml) was mixed with 300 ml of sterilized soil (total 1,200 ml), and returned to the original pot. Each pot then was replanted to one germinated 'Early Runner' peanut seed.

The test was terminated after about 9 months on October 19, 1967. The roots were freed from the soil, washed, and weights of the roots and tops were recorded along with the weight and number of nuts.

Parasitism on Strawberry

Strawberry "mother" plants were grown in the greenhouse and runners were allowed to root in plastic pots of a four-inch size containing sterilized soil. About 45 days later, 12 plants (one plant per pot) of a uniform size and shape were selected for the test. The plants then were transferred to six-inch plastic pots each of which contained 1,500 ml of sterilized soil. On December 26, 1967, the plants were inoculated with suspensions of the three sting nematode populations. Each treatment was replicated three times in a randomized block design.

The experiment was terminated after 4 months on April 21, 1968. Data were taken on weight of whole plants (root and shoot). Weight of the root system alone, and sting nematode recovery from 100 ml of soil.

Parasitism on Tomato

Tomato seeds were sown in sterilized soil in a wooden flat. Two of the resulting seedlings were transplanted to each of 35 plastic pots of a four-inch size filled with sterilized soil. Two weeks later, 24 of the pots were chosen for the test based upon uniformity of size and

condition of the tomato plants. The contents of each pot was transferred to a six-inch pot. Additional sterilized soil was added to make a total of 1,000 ml per pot. On December 26, 1967, plants were inoculated with suspensions of the respective sting nematode populations. Treatments were replicated three times and pots were arranged in a randomized block design.

The test was terminated after 5 months on June 2, 1968. Stems of the tomato plants were cut at the soil surface and the foliage weights recorded. The roots were freed from the soil, washed, surface dried with paper towels, and weighed. A 150 ml sample of soil was taken from each pot and the sting nematode counts were recorded.

Effects of Sting Nematode Parasitism on the Mineral Balance of Rough Lemon

As indicated earlier, a portion of the root system and 10 leaves were taken from each plant for mineral analyses. The samples were placed in paper bags, and dried at 70° F for 36 hours. All samples were ground in a Wiley Mill to pass through a 60-mesh screen.

Nitrogen determination was made with a micro-Kjeldahl apparatus using $\text{Cu SO}_4 \cdot \text{H}_2\text{O}$ as a catalyst (2). Ammonia was distilled into a boric acid solution and titrated with 0.1 N HCl.

The ash was digested with 5 N HCl, and analyzed according to the methods described by Jackson (41) as follows: K, Na and Ca were determined with the Beckman flame spectrophotometer; Fe, Mn and Mg by the double-beam-atomic-absorption-flame spectrophotometer; and P chemically by precipitation as phosphomolybdate.

Morphological Studies on the Three Populations of
Belonolaimus longicaudatus

Specimens of the three populations were isolated from soil around the roots of tomato plants (14). Permanent slides of females, males and larval stages were prepared as described by Seinhorst (83).

Morphological studies were made on 17 females of the Gainesville population, 19 of the Fuller's Crossing population and 16 of the Sanford population. Ten males from each population were studied.

RESULTS AND DISCUSSION

Parasitism Tests

Parasitism on Rough Lemon

Rough lemon seedlings inoculated with the Fuller's Crossing population of B. longicaudatus were noticeably shorter within two months than the seedlings inoculated with the Sanford and Gainesville populations and the uninoculated controls. After six months the differences were even more distinct. While the seedlings inoculated with the Sanford and Gainesville populations showed no significant morphological differences from uninoculated plants, those inoculated with the Fuller's Crossing population were significantly stunted in growth by the parasite. The fresh weights of the latter were 29% less than that of the uninoculated checks (Table 1). The roots showed the stubby root condition described by Christie et al. (13), in that the roots were very short and thick (Figures 2, 3). Populations of the Fuller's Crossing nematode increased an average of about 60-fold during the six months period (Table 1). Under the same conditions, however, the Gainesville and Sanford populations neither reproduced nor caused any detectable damage to the plants (Table 1).

When the rough lemon seedlings were transferred to larger pots (six months after the initial inoculation) those inoculated with the Fuller's Crossing population continued to exhibit the same morphological symptoms (Figures 1, 2) and injury (Table 2). While differences in

Table 1.--Effects of three populations of Belonolaimus longicaudatus on rough lemon seedlings six months after inoculation with 100 specimens.

	Gainesville	Fuller's Crossing	Sanford	Check
Weight of whole plant (gm)	85.50 ^a	56.67 ^b	73.83	79.83
Nematode count per pot (1,000 ml)	75	5,920	18	0

^aAverage of six replications.

^bSignificant at the 5% level.

Table 2.--Effects of three populations of Belonolaimus longicaudatus on rough lemon seedlings fourteen months after inoculation with 100 specimens.

	Gainesville	Fuller's Crossing	Sanford	Check
Weight of foliage (gm)	84.00 ^a	66.50	76.33	78.17
Weight of roots (gm)	81.50	64.83	81.50	83.83
Number of leaves	94.83	125.83 ^b	97.33	96.83
Dry weight of 10 leaves (gm)	2.75	1.65 ^c	2.28	2.73
Height of plants (inch)	40.67	24.50 ^b	32.17	33.17
Nematode count per pot (3,500 ml)	750	11,145	11	0

^aAverage of six replications.

^bSignificant at the 5% level.

^cSignificant at the 1% level.



Figure 1.--Comparison between representative rough lemon plants inoculated with Sanford (S), Gainesville (G), and Fuller's Crossing (FC) populations of Belonolaimus longicaudatus and the non-inoculated check plants (C) fourteen months after inoculation.

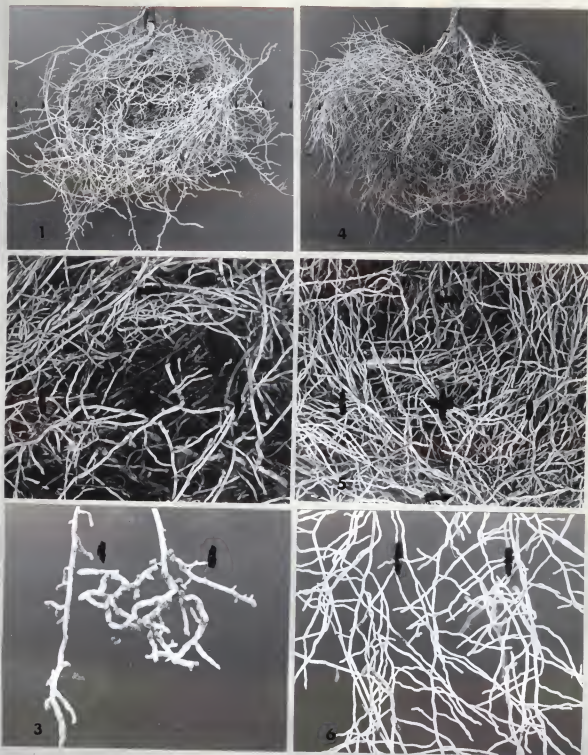


Figure 2.--Comparison between representative rough lemon root systems parasitized by the Fuller's Crossing population of Belonolaimus longicaudatus (1, 2, 3) and non-parasitized roots (4, 5, 6) fourteen months after inoculation.

fresh weight of foliage and roots of plants inoculated with the Fuller's Crossing nematodes were not statistically significant from the check plants, the averages were reduced 16.2 and 23.7%, respectively. Height of seedlings was significantly less than the check plants, and the plants were "bushy" with extensive lateral foliage proliferation. The leaves were rough, small in size, and statistically greater in number but less in weight than the checks. The leaves of infected plants maintained a normal green color throughout the experiment. The roots of rough lemon seedlings supported extremely high populations of the Fuller's Crossing population averaging 11,145 specimens per plant 14 months after inoculation (Table 2). The roots showed limited necrosis and were generally discolored.

Fourteen months after inoculation, the Gainesville population had increased an average of less than 8 times. A few of the roots exhibited the stubby root condition, but generally the plants appeared normal (Table 2). B. longicaudatus from the Sanford collection failed to reproduce on the roots of rough lemon seedlings and no disease symptoms were observed.

Parasitism on Peanut

Peanut plants inoculated with the three populations of B. longicaudatus showed no statistical differences from each other or from the uninoculated controls five months after inoculation. The average fresh weight of foliage and roots of plants inoculated with either one of the three populations showed no significant difference from that of the check plants (Table 3). In all cases the numbers of nematodes were low.

Table 3.--Effects of three populations of Belonolaimus longicaudatus on peanut plants five months after the first planting.

	Gainesville	Fuller's Crossing	Sanford	Check
Weight of foliage (gm)	21.53 ^a	21.65	23.61	24.33
Weight of roots (gm)	8.81	9.62	10.45	9.55
Nematode count per pot (1,000 ml)	76	133	165	0

^aAverage of six replications.

Table 4.--Effects of three populations of Belonolaimus longicaudatus on peanut plants four months after the second planting.

	Gainesville	Fuller's Crossing	Sanford	Check
Weight of foliage (gm)	16.33 ^a	18.17	16.67	24.50
Weight of roots (gm)	3.00 ^b	5.42	1.87 ^b	5.69
Nematode count per pot (1,000 ml)	373	177	317	0

^aAverage of six replications.

^bSignificant at 1% level.

Figure 3.--Comparison between peanut roots inoculated with the Fuller's Crossing (FC) and Sanford (S) population of Belonolaimus longicaudatus five months after the second planting.



Figure 4.--Comparison between peanut roots inoculated with the Gainesville (G) population of Belonolaimus longicaudatus and non-inoculated check (CK) roots five months after the second planting.



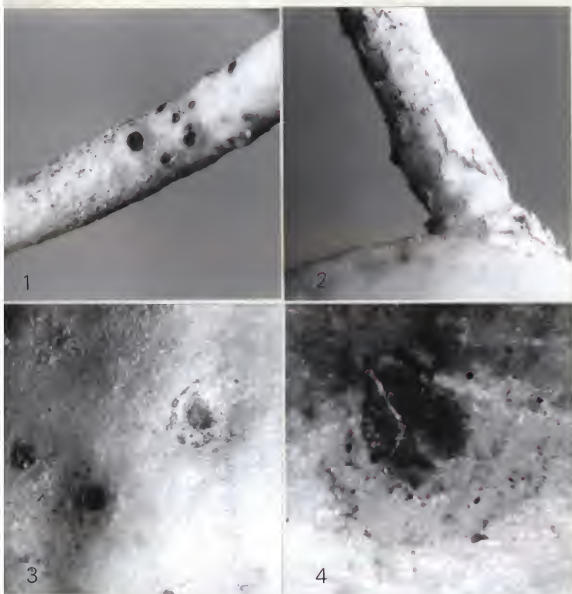


Figure 5.--Necrotic lesions on the pegs (1, 2) and hulls (3, 4) of peanut inoculated with the Sanford population of Belonolaimus longicaudatus.

In a second experiment, after four months, those plants inoculated with the Sanford and Gainesville populations were stunted and chlorotic with more severe damage caused by the Sanford population. Plants inoculated with the Fuller's Crossing population appeared to be healthy but the average weight of foliage was 26 percent less than that of the control plants (Table 4). Foliage weights of the plants inoculated with the Gainesville and Sanford populations were 33 and 32 percent less than the check plants (Table 4), but these reductions were not statistically significant. Reductions in the average root weights of plants which had been inoculated with the Sanford and Gainesville populations were 61 and 47 percent and were significant at the 1% level (Table 4). The average root weight of the plants inoculated with the Fuller's Crossing population was not significantly different from that of the control. The number of nematodes recovered were about 4, 3, and 2 times as many as the original inoculum in the Gainesville, Sanford and Fuller's Crossing populations, respectively (Table 4).

The greatest root damage occurred on plants inoculated with the Sanford population where roots were greatly reduced and discolored (Figure 3-S). Extensive necrosis appeared on the roots, pegs and hulls (Figure 5). The same symptoms, but less severe, were observed on the roots of those plants which had been inoculated with specimens of the Gainesville population (Figure 4-G). The Fuller's Crossing population caused little injury and sporadic necrosis on the roots.

The results of this experiment indicate that the Sanford population did parasitize peanuts and produce symptoms of injury even at low population levels. Apparently, the peanut plants were unable to support large numbers of the parasite, a situation comparable with

strawberry plants described by Christie et al. (13), who suggested, as a reason, failure of the parasitized plants to produce new roots. Perry and Norden (67) found that B. longicaudatus failed to attack peanut planted in a field near Gainesville. However, under the greenhouse conditions of this experiment, the Gainesville population was able to produce symptoms of injury to peanut plants. These differences may be due to ecological factors which were not defined. The Fuller's Crossing population apparently failed to establish a host-parasite relationship of any significance with peanut plants.

Parasitism on Strawberry

During a four months period, the growth of strawberry plants was affected by the Gainesville population of B. longicaudatus (Table 5). Although statistical analyses showed no significant differences from the check plants, the average weights of foliage and roots were reduced by 21 and 32.5 percent, respectively. The plants inoculated with the Gainesville population were stunted and the foliage was dull-looking and mildly chlorotic. The root systems were distinctly reduced and discolored and new roots were sparse and stubby. The nematode reproduced to increase the population by an average of 4-fold. Apparently, the strawberry plants were not able to support high populations of this parasite because as suggested by Christie et al. (13), the plants failed to produce secondary roots necessary for nematode feeding.

The Sanford and Fuller's Crossing populations failed to reproduce and inflicted no detectable injury to the inoculated plants (Table 5).

Parasitism on Tomato

Retardation of top growth of the tomato plants inoculated with the

Table 5.--Effects of three populations of Belonolaimus longicaudatus on strawberry plants four months after inoculation with 100 specimens.

	Gainesville	Fuller's Crossing	Sanford	Check
Weight of foliage (gm)	9.33 ^a	11.17	11.23	11.73
Weight of roots (gm)	2.63	3.80	3.40	3.90
Nematode count per pot (1,500 ml)	393	7	37	0

^aAverage of three replications.

Table 6.--Effects of three populations of Belonolaimus longicaudatus on tomato plants five months after inoculation with 100 specimens.

	Gainesville	Fuller's Crossing	Sanford	Check
Weight of foliage (gm)	72.33 ^a	62.67 ^b	84.00	86.33
Weight of roots (gm)	9.37 ^c	7.77 ^c	16.60	17.83
Nematode count per pot (1,000 ml)	8,552	10,190	2,960	0

^aAverage of three replications.

^bSignificant at the 5% level.

^cSignificant at the 1% level.



Figure 6.--Comparison between non-inoculated tomato roots (CK) and roots inoculated with the Fuller's Crossing (FC) population of Belonolaimus longicaudatus five months after inoculation.

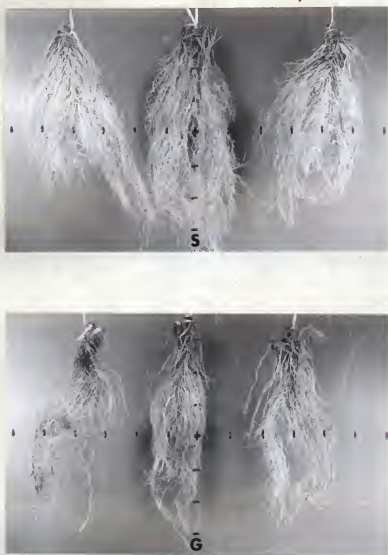


Figure 7.--Comparison between tomato roots inoculated with the Sanford (S) and Gainesville (G) populations of Belonolaimus longicaudatus five months after inoculation.

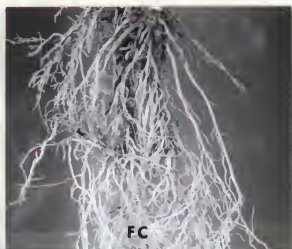


Figure 8.--A close-up of tomato roots inoculated with the Fuller's Crossing (FC) of Belonolaimus longicaudatus and uninoculated check (CK).

Fuller's Crossing population began to appear about two months after inoculation. During the next three months retardation in growth became more conspicuous and slight foliar chlorosis began to appear. Same symptoms showed on the plants inoculated with the Gainesville population but to a lesser extent.

At termination of the experiment, the average weight of the foliage of plants inoculated with the Fuller's Crossing, Gainesville and Sanford populations was reduced from that of the check plants by 27.3, 16.3 and 2.7%, respectively (Table 6). Reduction by the Fuller's Crossing population being statistically different at the 5% level of significance. Reduction in the average weight of roots inoculated with the Fuller's Crossing and Gainesville populations were 56 and 47.5% and was statistically significant at the 1% level. Weight of roots of plants inoculated with the Sanford population was reduced 7% but was not statistically significant (Table 6).

Roots of the plants inoculated with the Fuller's Crossing and Gainesville populations showed the stubby root condition and were discolored and necrotic (Figures 6-FC, 8-FC). No symptoms appeared on plants inoculated with the Sanford population (Figure 7-S). The tomato plants supported extremely high populations of B. longicaudatus with an average increase of 102, 85, and 30 times with the Fuller's Crossing, Gainesville, and Sanford populations, respectively.

Effects of Belonolaimus longicaudatus on the
Mineral Balance of Rough Lemon

Among the three populations of B. longicaudatus tested for parasitism to rough lemon plants, only the Fuller's Crossing population incited significant changes in the mineral levels of the leaves and

roots when compared with control plants (Table 7). The average rate of P was significantly lower in the diseased roots, which was in agreement with the results of certain host-parasite relationships (20, 95) but contrary to others (47, 48, 56, 57). K level was higher in the root samples of the diseased plants, which was contrary to the findings of other investigators (90, 95, 96). K was also higher in the leaf samples, contrary to the results of Feldman et al. (23) who reported a decrease in the rate of K in the leaves of declining citrus trees parasitized by Radopholus similis. The amounts of Na were significantly lower in the diseased roots, which was in agreement with the results of some workers (47, 48, 96) but contrary to the findings of Tarjan (90) on English boxwood parasitized by Pratylenchus spp. The amounts of Fe were significantly lower in the diseased leaf samples, which corresponded to the results of Chitwood et al. (9) on peach. The level of Mg was significantly higher in the diseased leaves but lower in the roots. These results were in agreement with the findings of others (4, 48, 95, 96). The leaves of diseased plants showed significantly lower rates of Mn. No differences in the levels of N and Ca were found in the roots or leaves of the parasitized plants. Previous workers have reported either a decrease (52, 96) or an increase (4, 47, 48, 90) in the levels of N in the root samples, and others (95, 96) reported a decrease in Ca rates in the diseased roots.

Rough lemon plants inoculated with the Gainesville population of B. longicaudatus resulted only in minor differences from those of the check plants (Table 7); lower rates of Fe in the leaf samples, higher rates of Mg in the leaf samples, and lower rates of Mg in the roots. In the samples taken from the leaves and roots of rough lemon plants

Table 7.--Effects of three populations of Belonolaimus longicaudatus on the mineral levels of the leaves and roots of rough lemon plants fourteen months after inoculation with 100 specimens.

		Mineral levels in ppm.			
		Gainesville	Fuller's Crossing	Sanford	Check
N	Leaves	14,617 ^a	14,645	14,159	13,813
	Roots	19,613	21,044	21,100	20,418
P	Leaves	1,943	1,862	1,862	1,630
	Roots	2,930	2,073 ^c	3,020	3,533
K	Leaves	10,100	15,550 ^c	11,100	8,970
	Roots	11,550	14,917 ^b	12,845	11,950
Na	Leaves	965	1,355 ^b	1,180	1,055
	Roots	6,150	4,317 ^b	5,463	5,333
Ca	Leaves	27,233	28,600	29,283	33,500
	Roots	1,072	948	978	957
Fe	Leaves	55 ^b	61 ^b	85	81
	Roots	461	416	351	411
Mg	Leaves	442 ^c	401 ^b	403 ^c	349
	Roots	594 ^b	455 ^c	561 ^c	708
Mn	Leaves	27	23 ^b	29	28
	Roots	1,067	742	872	1,222

^aAverage of six replications.

^bSignificant at the 5% level.

^cSignificant at the 1% level.

inoculated with the Sanford population, only higher levels of Mg in the leaves and lower levels in the roots were found (Table 7).

The results presented above indicated that the Fuller's Crossing population of B. longicaudatus caused definite changes in the normal balance of certain minerals in the leaves and roots of diseased rough lemon plants. Mg and Fe comprised the sole mineral changes in the leaves and roots of the plants inoculated with the Gainesville or Sanford populations.

Morphological Studies on Three Populations of
Belonolaimus longicaudatus

Average total lengths of females from the Gainesville, Fuller's Crossing, and Sanford populations of B. longicaudatus were 2,097, 2,274, and 2,206 microns, respectively (Table 8). These measurements were not significantly different. In each case they ranged between the average lengths of B. gracilis (2,014 microns) and B. longicaudatus (2,500 microns) as indicated by Rau (71), but corresponded to measurements reported by Graham and Holdeman (30). The average stylet lengths of females of the three populations were not significantly different (Table 8). They corresponded to lengths reported by Graham and Holdeman (30) but were much less than reported for B. gracilis by Rau (71).

The females of the Sanford population showed a lower average "c" value than that of the Gainesville and Fuller's Crossing populations (Table 8). This was due to variations in the length of the tails which averaged 119, 134, and 141 in the Gainesville, Fuller's Crossing, and Sanford specimens, respectively. The number of annules on the females tails varied also (Table 8). The average "a" value was higher in females of the Fuller's Crossing population than in the Gainesville

Table 8.--Morphological data^a on the females of Belonolaimus longicaudatus from Gainesville (G), Fuller's Crossing (FC) and Sanford (S).

	G	FC	S
Total length	2,097	2,274	2,206
a	52	64	59
b	7.5	7.9	7.8
c	17.2	17.6	14.9
V	51.7%	49.9%	49.7%
Number of annules on head	7.4	7.8	7.5
Stylet length	111	118	106
Prorhabdion length	88	85	81
Meso - and metarhabdion length	23	33	23
Anterior end - Guiding ring	60	55	54
Anterior end - Median bulb	185	189	179
Length of median bulb	27	31	27
Width of median bulb	22	21	21
Anterior end - Excretory pore	223	217	224
Anterior end - Base of esophagus	279	287	281
Anterior end - Vulva	1,089	1,144	1,098
Vulva - Terminus	1,009	1,145	1,107
Length of anterior gonad	416	459	496
Length of posterior gonad	390	431	491
Width at vulva	41	36	38
Width at anus	34	30	33
Anus - Phasmid	37	42	49
Phasmid - Terminus	82	88	92
Tail length	119	131	141
Number of annules on tail	68	82	94

^aAverages of 17, 19 and 16 females from the Gainesville, Fuller's Crossing and Sanford, respectively. Measurements in microns.

Table 9.--Morphological data^a on 10 males of Belonolaimus longicaudatus from Gainesville (G), Fuller's Crossing (FC), and Sanford (S).

	G	FC	S
Total length	1,712	1,790	1,718
a	49	64	56
b	6.5	6.9	6.9
c	14.8	14.6	14.1
Stylet length	115	107	106
Length of meso - and metarhabdion	32	29	29
Anterior end - Median bulb	209	167	173
Length of median bulb	25	22	23
Width of median bulb	19	17	17
Anterior end - Basal bulb	263	258	249
Anterior end - Excretory pore	215	200	203
Greatest body width	35	28	31
Width at cloaca	24	22	24
Spicules length	53	43	42
Gubernaculum length	19	17	17
Length of gubernaculum flexure	7.2	6.6	5.6
Gonad length	689	685	733
Tail length	116	122	122

^aMeasurements in microns.

or Sanford populations indicating a narrower body width at the vulva. The average "a" value of the Sanford population was intermediate (Table 8).

The average "a" values of the males followed the same trend as the females (Table 9). Average length of spicule, gubernaculum and gubernaculum flexure of the males of the Gainesville population gave higher values than those of the other two populations.

CONCLUSIONS

The three populations of Belonolaimus longicaudatus were found to constitute three different physiological races based on the following:

1. The Fuller's Crossing population reproduced well and caused injury to rough lemon and tomato but not to strawberry and peanut plants.
2. The Gainesville population reproduced well and caused injury to peanut, strawberry and tomato but not to rough lemon plants.
3. The Sanford population reproduced well and caused injury to peanut, reproduced well on tomato, but neither reproduced nor caused injury to strawberry and rough lemon plants.
4. Parasitism of the Fuller's Crossing population on rough lemon plants resulted in an increase of P, K and Mg in the leaves; low rates of Fe and Mn in the leaves; high level of K in the roots; and low rates of P, Mg, and Na in the roots. No effect on the mineral balance was found in the leaf and root samples taken from plants inoculated with specimens of the Sanford and Gainesville populations.
5. Females of the Sanford population had a lower "c" value and greater number of annules on the tails than females of the Gainesville and Fuller's Crossing populations. Females and males of the Fuller's Crossing population had higher "a" values than those of the other two populations.

SUMMARY

Three populations of Belonolaimus longicaudatus from Gainesville, Fuller's Crossing, and Sanford were tested for parasitism on citrus (rough lemon), peanut (Early Runner), strawberry (Florida 90), and tomato (Rutgers).

The rough lemon seedlings supported high populations of the Fuller's Crossing nematodes. The above ground portions of the parasitized plants were stunted and bushy. The leaves were rough, small in size but more numerous as compared with the control plants. The foliage of the diseased seedlings showed no chlorosis. The root systems showed a "stubby root" condition, where the lateral roots were sparse, short and thick. Specimens from the Gainesville and Sanford populations failed to establish themselves on rough lemon and no differences were detected in the condition of the roots or foliage between the inoculated and check plants.

The peanut plants were severely injured by the Sanford population. The foliage was stunted and chlorotic and the roots, pegs and hulls were necrotic and discolored. The Gainesville population had the same effects on the peanut plants as those exhibited by the Sanford population but to a lesser extent. The Fuller's Crossing population produced some necrosis on the roots but it had no effects on the general condition of the plants.

Tomato plants supported very high populations of the Fuller's

Crossing and Gainesville nematodes. The foliage was stunted and chlorotic, and the roots were stubby, short, and exhibited very limited lateral proliferation. However, tomato plants supported relatively low populations of the Sanford nematodes and no injury to the plants was detected.

On strawberry, the Sanford population was able to establish itself to a limited extent as compared with the Gainesville population. The root systems were reduced, stubby and discolored. The Fuller's Crossing population, however, was not able to maintain itself on the strawberry roots.

The leaves and roots of the B. longicaudatus inoculated and not inoculated rough lemon plants were analysed for mineral contents. The leaves of the plants parasitized by the Fuller's Crossing population had higher rates of P, K, and Mg, but lower rates of Fe and Mn in comparison with the leaves of the check plants. The roots of the diseased plants had high levels of K, but low rates of P, Mg, and Na. However, no significant differences were found between the nematode parasitized and check plants either in the levels of N, Ca and Na in the leaves or in the levels of N, Ca, Mn and Fe in the roots.

Variations in the morphological characteristics among the three populations of B. longicaudatus included:

1. Females of the Sanford population had a lower "c" value than females of the other two populations, due to longer tails of the former. Females of the Sanford population also had a higher number of annules on the tail than did females of the Gainesville population, while those of the Fuller's Crossing were intermediate.

2. The average "a" values of the males and females of the Fuller's Crossing population were higher than those of the Gainesville population indicating larger body width at the vulva of the latter. The "a" values of the males and females of the Sanford population were intermediate.
3. Average length of spicule, gubernaculum, and gubernaculum flexure of the Gainesville population tended to be greater than those of the other two populations.

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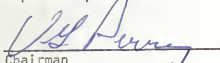
This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

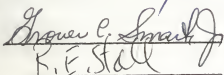
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